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Hormonal responsiveness in the Trier Social Stress Test and the dexamethasone-corticotropin releasing hormone test in healthy individuals

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Abstract: A number of different laboratory procedures investigate the hormonal response in a standardized pharmacological challenge test (dexamethasone-corticotropin releasing hormone; DEX-CRH) or in a psychosocial stress induction on the hypothalamic–pituitary–adrenocortical axis by the Trier Social Stress Test (TSST). However, the magnitude of the response related to the different stressors and the interaction of the responsiveness between the two tests is still unclear. Fifty-two participants underwent both the DEX-CRH test and the TSST on two separate days. The cortisol and the plasma adrenocorticotropic hormone (ACTH) release were assessed before and after the stress tests. For a specification of the cortisol response to both conditions, subgroups of high- and low-cortisol responders to the TSST and the DEX-CRH test were formed. The healthy participants showed a substantial increase in the ACTH and the cortisol concentration after the two tests. This increase was 3 times greater in the TSST than the DEX-CRH test. High responders in both tests demonstrated a higher factor of the cortisol reactivity ratio (TSST/DEX-CRH test). Psychosocial stress as induced by the TSST was associated with a significantly greater increase in cortisol compared to the DEX-CRH test, even though the ACTH response displayed no differences. Our findings indicate an interaction of the hormonal responsiveness between the two tests with regard to the cortisol patterns.

Keywords: adrenocorticotropic hormone (ACTH); cortisol; dexamethasone-corticotropin releasing hormone (DEX-CRH) test; hypothalamic–pituitary–adrenocortical (HPA) axis; Trier Social Stress Test (TSST)

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Approximately 450 million people worldwide suffer from stress or mental disorders (World Health Organization, 2001). From 1996 to 2006, health costs for stress or mental disorder in the United States rose from $35.2 billion to $57.5 billion dollars (Soni, 2009). Research on chronic stress and stress-related disorders such as anxiety disorders, depression, and posttraumatic stress disorder (PTSD) have revealed a hypothalamic–pituitary–adrenocortical (HPA) axis dysfunction (Tsigos, Kyrou, Kassi, & Chrousos, 2000). To understand the development of this dysfunction, experimental paradigms such as the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993a) and the dexamethasone-corticotropin releasing hormone test (DEX-CRH test) are used to evaluate the HPA axis.

For the purpose of investigating responses of the HPA axis, one distinguishes between exogenous and endogenous procedures. Due to the artificial synthesis of the corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) regulator peptides involved, the DEX-CRH test is considered as an exogenous procedure

and represents a reproducible, pharmacological challenge test. Aside from the direct influence on the biosynthesis, the effect of the hormones on its target organs is modified by glucocorticoids. Therefore, the stimulatory influence of the corticotropic cells of the frontal flaps of the pituitary gland (hypophysis) becomes weaker under the influence of dexamethasone (Eberwine, Jonassen, Evinger, & Roberts, 1987). As the reactivity of the HPA axis is significantly reduced by the induction of dexamethasone (Luo, Kiss, Rabadan-Diehl, & Aquilera, 1995), it seems to enable a positive regeneration of CRH via CRHI receptors on its own biosynthesis. This effect might represent a possibility for CRH-containing neurons of the nucleus paraventricularis to remain activated during long-term stress phases, thus keeping the reactivity of the HPA axis intact. The state of research on this effect, however, is inconsistent (Makino, Hashimoto, & Gold, 2002). All in all, the DEX-CRH test is the most sensitive procedure for testing the regulation ability of the HPA axis (Ising et al., 2007). Compared to other tests for the evaluation of the neuroendocrine course and the dysregulation of disorders, the DEX-CRH test proves to be more powerful in detecting even subtle changes in the ACTH and the cortisol concentration (Holsboer, 2001; Ising et al., 2005; Pariante, Thomas, Lovestone, Makoff, & Kerwin, 2004; Schreiber, Lauer, Krumrey, Holsboer, & Krieg, 1996). Beyond that, the DEX-CRH test is rather robust regarding disease-unrelated factors such as smoking, weight, and age (Künzel et al., 2003). In recent years, the DEX-CRH test has been studied extensively concerning psychopathology and stress. In major depression, the neuroendocrine response to the DEX-CRH test is elevated, but tends to normalize after response to antidepressant treatment, and a favorable response to antidepressant treatment can be predicted by determining the dexamethasone suppressor status on admission (Hennings et al., 2019; Ising et al., 2005). Chronically abused borderline patients show a significantly enhanced corticotropin (ACTH) and cortisol response to the DEX-CRH challenge compared with subjects who were not abused (Rinne et al., 2002). In panic disorder, patients with a higher cortisol release in the DEX-CRH test show less improvement after cognitive behavioral therapy (CBT) (Wichmann, Bornstein, Lorenz, & Petrowski, 2018). In summary, an elevated response to the DEX-CRH challenge proves to be a potent marker for a specific mental health vulnerability.

Because psychosocial stress plays a significant role in the development of mental disorders, the effect of acute psychosocial stress on the HPA axis represents an important component in stress research, as such investigated by so-called *endogenous procedures*. The Trier Social Stress Test counts as an internationally established protocol employed with both healthy individuals as well as patients (Kudielka, Hellhammer, & Kirschbaum, 2007). The experimental paradigm combines uncontrollability, social evaluation, and arithmetic tasks. In numerous studies, elevated cortisol concentrations induced by the TSST could be observed as a sign of a successful activation of the HPA axis in healthy individuals. A meta-analysis of 208 laboratory tests (Dickerson & Kemeny, 2004) showed that the TSST is one of a few stress tests capable of reaching a reliable cortisol response. The processing of psychosocial stressors requires the participation and evaluation by the higher brain centers, in contrast to the pharmacological test in which the substances have a direct effect on the target organ.

The hypothesized differences in the hormonal response patterns are based on the assumption that the psychosocial stress-response to the TSST embraces the activation of a complex stress matrix induced by cognitive evaluation processes involving autonomic as well as central nervous system structures (Hellhammer, Wüst, & Kudielka, 2009) whereas the neuroendocrine response to the DEX-CRH test underlies a physiological response pattern of the HPA axis, including feedback loops largely controlled by glucocorticoid receptor sensitivity (de Kloet, Joëls, & Holsboer, 2005; Leistner & Menke, 2018), not involving higher order processes.

There is growing evidence that a vulnerability to stress is associated with a dysregulation of the HPA axis. The investigation of the HPA axis by combining and comparing a pharmacological and a psychosocial stress test enables a comprehensive investigation of the HPA axis involving, on one hand, fundamental neuroendocrinological feedback loops and, on the other hand, the aforementioned higher order processes and possible interactions. This approach facilitates the understanding of the specific stress regulation mechanisms of the HPA axis in healthy subjects, which is the topic of the current study, as well as in psychiatric disorders.

In recent years, specific patterns of a dysregulation of the HPA axis found in stress-related psychiatric entities such as major depression and panic disorder by means of the DEX-CRH test and TSST. In major depression, there is growing evidence for an elevated neuroendocrine response to the DEX-CRH test, which tends to normalize after responding to antidepressant treatment (Hennings et al., 2019; Leistner &

Menke, 2018). However, in the TSST, patients with major depression show an equal or higher neuroendocrine response compared to healthy controls (Wichmann, Kirschbaum, Böhme, & Petrowski, 2017; Young, Abelson, & Cameron, 2004). In contrast, panic disorder patients typically display a blunted neuroendocrine response in the TSST (Petrowski, Wintermann, Schaarschmidt, Bornstein, & Kirschbaum, 2013) as well as a blunted cortisol response in the DEX-CRH test compared to healthy controls (Petrowski, Wintermann, Kirschbaum, & Bornstein, 2012). These findings support the crucial significance of a compromised glucocorticoid receptor sensitivity in major depression, and the pivotal relevance of higher order cognitive processes in panic disorder.

The aim of this study was to compare the two different challenges, TSST and DEX-CRH test, in healthy subjects to specify the magnitude and the time line of psychosocial and pharmacological stress induction. Previous research on the HPA axis activation by the TSST and the CRH test has shown similar maximums in cortisol concentration. However, the maximum peak was reached sooner in the TSST so that different temporal patterns could be observed (Kirschbaum, Wüst, Faig, & Hellhammer, 1993b). Against this backdrop, we hypothesized that healthy subjects show a greater increase in hormonal parameters after psychosocial stress induction compared to DEX-CRH injection, whereby the ratio of increase between both tests gives further insight into the underlying processes.

Furthermore, the association between the hormones responsiveness to the TSST and the suppression in the DEX-CRH test was investigated. Despite targeting different mechanisms in stress reactivity, it is not clear in which way there might be an association between the centrally induced stress reactivity and the attenuation of stress responsiveness via glucocorticoid feedback. Thus, we hypothesized that high responsiveness to the psychosocial stressor in the TSST corresponds to a low responsiveness to the feedback inhibition in the DEX-CRH test, and vice versa.

Materials and methods

Participants

The healthy participants for the study were recruited through newspaper advertisements and a notice board at the Technical University of Dresden. Exclusion criteria (organic and psychological diseases, etc.) were inquired about in a prior telephone interview. The study protocol only allowed for participants aged 18 to 65 years who smoked no more than 10 cigarettes/day and whose body mass index did not exceed 27 kg/m^2. The sample consisted of N = 52 healthy individuals, whose characteristics are described in Table 1. Mean age of the participants was 32.06 years (SD = 11.63) and included 29 females and 23 males. All participants were informed in detail about the targets of the study and declared their consent in written form. After successful participation, the test participants received an allowance of 50 euros. The study protocol was approved by the local Ethics Committee of the Medical Faculty of the Technical University of Dresden, Germany (No. EK46032008).

Table 1
Characteristics of the Participants

	Healthy participants
Total, N	52
Females, n	29
Males, n	23
Age (years), M (SD)	32.06 (11.63)
BMI (kg/m^2), M (SD)	23.43 (2.56)
Smoker, n (%)	12 (23.08)
Contraceptives, n (%)	13 (44.83)
$PASA_{PRIMARYAPPRAISAL}$, M (SD)	3.74 (0.84)
$PASA_{SECONDARYAPPRAISAL}$, M (SD)	4.27 (0.73)
$PASA_{STRESSINDEX}$, M (SD)	−0.54 (1.36)
STAI-$State_{PRE}$, M (SD)	43.56 (5.15)[a]
STAI-$State_{POST}$, M (SD)	42.20 (5.62)[a]

Note. BMI = body mass index; PASA = Primary Appraisal Secondary Appraisal; STAI = Spielberger State–Trait Anxiety Inventory.
[a] subsample of n = 50 participants.

Procedures

All the healthy individuals completed the DEX-CRH test and the TSST on separate days over a time frame of 1 month. Considering the circadian rhythm of the cortisol release during the day, the start of the two tests was limited to a time window from 2:00 p.m. to 2:30 p.m. so that the entire measurement was completed by 6:00 p.m. Blood samples were collected at all measuring points by an intravenous cannula. The cannula was inserted 60 min before the start of the stress induction to avoid a pain-induced cortisol release. Female participants were tested in the luteal phase to standardize the influence of the menstrual cycle. Figure 1 shows the measuring points of the blood samples for the cortisol determination of both tests.

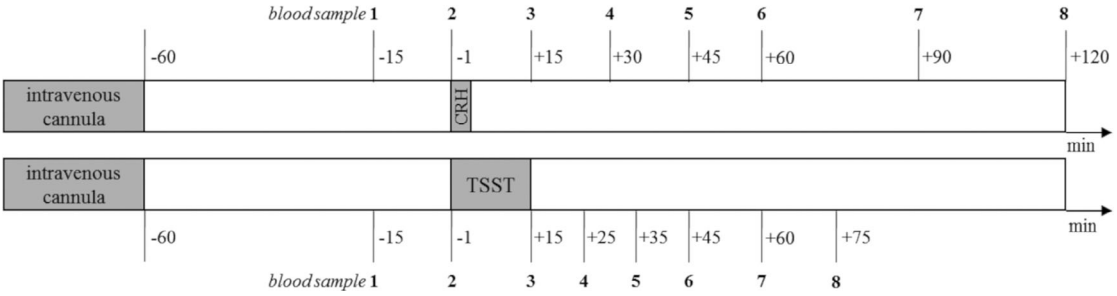

Figure 1. Measuring points of the blood samples for cortisol determination of the dexamethasone-corticotropin releasing hormone test (DEX-CRH test) and the Trier Social Stress Test (TSST).

DEX-CRH test

All participants were scheduled for the DEX-CRH test according to the procedure published by Schreiber et al. (1996) and Heuser, Yassouridis, and Holsboer (1994). An oral dosage of 1.5 mg dexamethasone was administered at 11:00 p.m. Subjects arrived for the test at 2:00 p.m. the following day. They were asked to refrain from eating, drinking, and smoking at least 2 hr before testing and during the 3½ hr of testing. The subjects rested in a comfortable, supine position with light reading permitted. A venous catheter was placed at 2:15 p.m. to later collect eight consecutive blood samples for the analysis of the ACTH and the cortisol concentration, respectively. After an accommodation time of 45 min, two blood samples were taken before an intravenous injection of 100 μg CRH was administered at 3:01 p.m. Afterward, six more blood samples were collected at 15-min intervals.

TSST

The TSST was performed according to the published process protocol by Kirschbaum, Pirke, and Hellhammer (1993a). The State Anxiety Inventory (STAI) was filled out once before and after the TSST. To assess the subjective stress perception, the Primary Appraisal Secondary Appraisal (PASA) was performed at the beginning of the TSST and the visual analog scale (VAS) immediately after the psychosocial stress inductions. The test team consisted of one male and one female wearing a white doctor's coat. Each gave instructions to a test participant of the opposite gender, to whom they had been introduced as psychologists trained in the analysis and evaluation of nonverbal body language. During the TSST, both test team members behaved deliberately cool and reserved. Verbal interactions between the test participants and the test team were kept at a minimum.

The test participant was invited to take part in a mock job interview. He or she also was informed in advance that the interview would be recorded by video camera and microphone for later analysis of voice and body language. After a preparation time of 5 min, in which the opportunity to prepare for the interview and to complete the questionnaire for subjective stress assessment (PASA) was provided, a 5-min interview before the test committee was carried out. After the 5-min job interview, there was an arithmetic task in which the test person subtracts in steps of 17 from the starting number 2,043. Possible errors are indicated by the test team, and the participant requested to start anew from 2,043. After 5 min, the calculation task was stopped, and the participant asked to leave the test chamber; outside, he was met by the test team member and accompanied back to the resting room. After the completion of the experimental protocol, an exhaustive and detailed elucidation on the fictional character of TSST situation was carried out.

ACTH and cortisol analysis

For ascertainment of the plasma ACTH concentrations, blood samples were collected into tubes containing trasylol and ethylenediaminetetraacetic acid (EDTA) (Sarstedt, Nümbrecht, Germany). For the specification of the cortisol concentrations, blood samples were collected into tubes called Serum-Gel-Monovette (Sarstedt, Nümbrecht, Germany). Blood was cooled at 4 °C immediately after sampling. After testing, blood specimens were centrifuged for 10 min at 4 °C and 3,000 rpm. After aliquoting, the plasma was stored at −80 °C and at −20 °C before being assayed for ACTH and cortisol, respectively. Plasma cortisol concentrations were analyzed using a commercially available radioimmunoassay kit by the Solid Phase Antigen Linked Technique (SPALT) with the LIAISON-Analyzer (DiaSorin, S.p.A., Italy). The detection limit was <4.14 nmol/L plasma; intra-assay coefficient of variation for 55 nmol/L and

386 nmol/L levels were ≤ 4%; interassay coefficient of variation <10%. Plasma ACTH was measured by immunoradiometric assay (Immulite, 2,500 ACTH, Erlangen, Germany). The detection limit for plasma ACTH concentrations was 1.1 pmol/L, and the intra- and interassay coefficients of variation at 4.4 pmol/L plasma were ≤ 5%.

Psychological assessments

The psychological stress and the perceived anxiety from the TSST were measured by two instruments: (a) The PASA (Gaab, 2009), consisting of 16 items with a 6-point rating scale (1 '*Strongly disagree*' to 6 '*Strongly agree*'), was used to evaluate the Stress-Index. Reliabilities. Cronbach's α in the current sample for the primary scales were α = 0.85 (threat), α = 0.51 (challenge), α = 0.87 (self-concept), and α = 0.72 (control expectancy). (b) Based on 20 items with a 4-point rating scale (1 '*Not at all*' to 4 '*Very much so*'; range = 20–80), the state anxiety was measured by the State–Trait Anxiety Inventory (STAI; Laux, Glanzmann, Schaffner, & Spielberger, 1981; Spielberger, Gorsuch, & Lushene, 1970). The reliability (Cronbach's α) in the current sample for the subscale State Anxiety of the STAI was α = 0.72.

Statistical analysis

To test the first hypothesis, analyses of variance (ANOVAs) for repeated measures were performed. A sensitivity power analysis (G*Power Version: 3.1.9.2, Faul, Erdfelder, Lang, & Buchner, 2007) showed that based on two testing conditions (DEX-CRH vs. TSST), in each condition n = 6 repetitions, a significance level of p = .05, power of 80% ($1-\beta$ = 0.80), and a total sample size of N = 52 subjects, the minimal detectable effect size of η^2 = .021 (critical F = 2.250) could provide. To test the second hypothesis, dependent Student t tests were performed. Based on a significant level of p = .05, power of 80% ($1-\beta$ = 0.80), and a total sample size of N = 52, the minimal detectable effect size of d = .35 (critical t = 1.675) could provide (G*Power Version: 3.1.9.2). Data were analyzed according to the normality of distributions and were, in case of not normally distributed data, subjected to natural log transformations.

First, the effects of the hormonal induction (CRH) and moderate psychological stress (TSST) over six measurement points were analyzed by the ANOVA for repeated measurements to reveal possible main effects of test or time and a possible Test × Time interaction for the cortisol and the ACTH. Because the standardized measurements time points of both tests differed, one value for the TSST (+30) and the DEX-CRH test (+75) was calculated by linear interpolation (Holling & Gediga, 2011) to have six concurrent time points for the analysis. The degree of freedom was adjusted with the Greenhouse–Geisser approach, taking sphericity into account.

Second, to reflect the effects of both tests on the HPA system, the area under the response curve (AUC) with respect to increase for ACTH and cortisol (AUC_I), and the delta between peak (either baseline, +15-, +30-, +45-, +60-, +75-min sample) and baseline (Δ Peak Base) were calculated (Fekedulegn et al., 2007). To compare the calculated values of the DEX-CRH test and TSST, the t test for dependent means was used.

Third, for the specification of the interaction between both stress tests, subgroups were formed by a median split of cortisol reactivity (AUC_I) with regard to the response in the TSST and in the DEX-CRH test. In a first round, the 52 participants were categorized into low ($AUC_I \leq$ 5.28 nmol/L*min, n = 26) and high responders ($AUC_I >$ 5.28 nmol/L*min, n = 26) for the TSST. In a second round, the 52 participants were categorized into low ($AUC_I >$ 0.96 nmol/L*min, n = 26) and high responders ($AUC_I \leq$ 0.96 nmol/L*min, n = 26) for the DEX-CRH test. Consequently, it could be possible for a subject from the high responders of the TSST to be categorized to the low or high responders of the DEX-CRH, and vice versa. The t test for dependent means was performed separately for the low and high responder groups in the TSST and DEX-CRH test to evaluate the difference between both tests in the cortisol and ACTH reactivity (AUC_I). Additionally, to take into account the fact of multiple testing, the Bonferroni–Holm correction was used.

Fourth, the chi-square test was performed to test the overlap between the subgroups of the low and high responders of the TSST and DEX-CRH test. In addition, differences between the subgroup of TSST high responders/DEX-CRH low responders (n = 15) compared to the subgroup DEX-CRH high responders/TSST low responders (n = 15) were tested by the independent t test.

Results

The ACTH and the cortisol concentration in response to the TSST showed higher values during all measuring

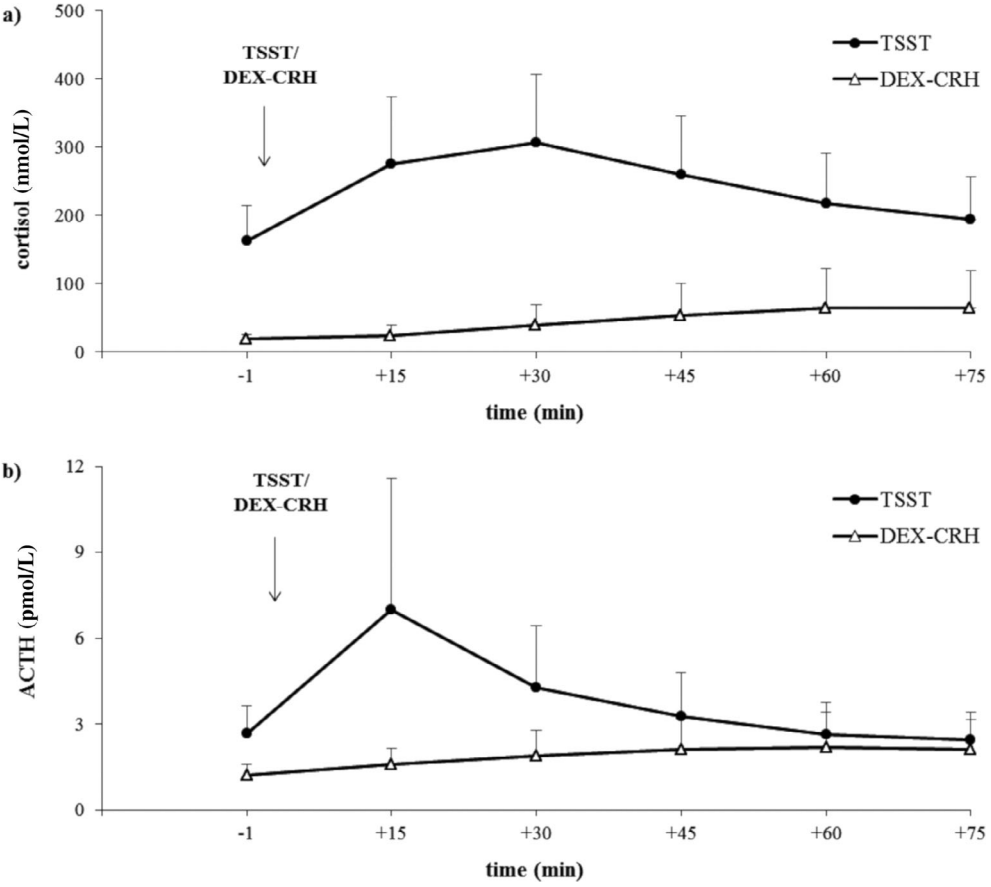

Figure 2. TSST and DEX-CRH test induced cortisol (a) and ACTH (b) response ($M \pm SD$) in healthy participants. DEX-CRH = dexamethasone-corticotropin releasing hormone test; TSST = Trier Social Stress Test.

points, as compared to the DEX-CRH test (Figure 2). These differences were highly significant between the two tests in the ACTH, $F(1, 51) = 97.311$, $p < .001$, $\eta^2 = .66$, and cortisol concentration, $F(1, 51) = 306.869$, $p < .001$, $\eta^2 = .86$, over the six measurement points with a highly significant Effect Time x Test interaction, ACTH: $F(2.638, 134.515) = 92.440$, $p < .001$, $\eta^2 = .65$; cortisol: $F(2.281, 134.515) = 56.115$, $p < .001$, $\eta^2 = .52$. In addition, the cortisol and ACTH of the healthy participants needed a longer time from baseline to peak after the induction of CRH, as compared to the TSST (see Figure 2).

Regarding the AUC_I, a significant difference between the tests could be unveiled for cortisol, AUC_I: $t(51) = 4.732$, $p < .001$, $d = 1.00$, but not for ACTH, AUC_I: $t(51) = -1.240$, $p = .22$. The AUC_I for the cortisol concentration of the TSST showed higher values in comparison to the DEX-CRH test (Table 2).

The delta between peak and baseline (Δ Peak Base) for ACTH and cortisol also differed significantly between the two tests, ACTH Δ Peak Base: $t(51) = 2.832$, $p < .01$, $d = 0.59$; Cortisol Δ Peak Base: $t(51) = 6.052$, $p < .001$, $d = 1.29$. The baseline to peak values of ACTH and cortisol increased sharper after the TSST, as compared to the DEX-CRH test (Table 2).

When analyzing the cortisol reactivity of subgroups as defined by the response to TSST and following the DEX-CRH test, there were highly significant differences between the two tests in the subgroups of TSST high responders, Cortisol AUC_I: $t(25) = 11.413$, $p < .001$, $d = 3.09$, and DEX-CRH low responders, Cortisol AUC_I: $t(25) = 8.012$, $p < .001$, $d = 2.23$. In contrast, results of the subgroups of TSST low responders and DEX-CRH high responders showed no difference in cortisol reactivity (Tables 3 and 4). Regarding ACTH reactivity, the TSST low responders, ACTH AUC_I: $t(25) = -2.914$, $p < .05$, $d = -0.91$, and DEX-CRH high responders, ACTH AUC_I: $t(25) = -3.490$, $p < .01$, $d = -0.97$, exhibited significant differences between the two tests. In contrast, results of the subgroups of DEX-CRH low responders and TSST high responders demonstrated no difference in ACTH activity (Tables 3 and 4). There were no significant differences in the baseline cortisol before bolus injection of 100 µg CRH between DEX-CRH low and high responders, $t(50) = 0.744$, $p = .46$, as well as between TSST low and high responders,

Table 2
Area under the Curve with Respect to Increase and Delta Values *(Δ)* for Cortisol and ACTH in Healthy Participants in the TSST and the DEX-CRH Test

	M (*SD*)			
	TSST	DEX-CRH	*t*(51)	*p*
Cortisol AUC_I	419.48 (380.35)	128.64 (158.47)	4.732	<.001*** (*d* = 1.00)
ACTH AUC_I	6.38 (7.92)	3.35 (4.12)	−1.240[a]	.22[a]
Cortisol Δ Peak Base	157.80 (102.83)	50.26 (57.62)	6.052	<.001*** (*d* = 1.29)
ACTH Δ Peak Base	4.33 (4.22)	1.10 (1.25)	2.832[a]	<.01**[a] (*d* = .59)

Note. Δ = delta values; AUC_I = area under the curve with respect to increase; DEX-CRH = dexamethasone-corticotropin releasing hormone test; TSST = Trier Social Stress Test.
[a] Calcuation based on logarithmized data. ** $p < .01$. *** $p < .001$.

Table 3
Area under the Curve with Respect to Increase for Cortisol and ACTH of Low/High TSST-Responders in the TSST and the DEX-CRH Test

		M (*SD*)				
		Cortisol AUC_I TSST	Cortisol AUC_I DEX-CRH	*t*(25)	*p*	Factor: TSST to DEX-CRH
TSST-Responder (*n* = 52)	Low (*n* = 26)	114.31 (209.81)	150.73 (166.11)	−0.582	.565	1
	High (*n* = 26)	724.65 (239.54)	106.56 (150.40)	11.413	< .001*** (*d* = 3.09)	7
		ACTH AUC_I TSST	ACTH AUC_I DEX-CRH			
TSST-Responder (*n* = 52)	Low (*n* = 26)	1.80 (3.39)	3.65 (4.27)	−2.914	< .01**[a] (*d* = −.91)	0.5
	High (*n* = 26)	10.97 (8.52)	3.05 (4.01)	1.369	.366[a]	4

Note. AUC_I = the area under the curve with respect to increase; DEX-CRH = dexamethasone-corticotropin releasing hormone test; TSST = Trier Social Stress Test.
[a] Calcuation based on logarithmized data. ** $p < .01$. *** $p < .001$.

$t(50) = 0.353$, $p = .73$. Additionally, in the groups of high and low responders for both tests, there were no differences regarding sex distribution, age, smoking, and intake of contraceptive pills (data not shown).

Concerning the anticipatory cognitive appraisal of the TSST, participants showed similar values in the four primary scales of the PASA (Threat: $M = 3.19 \pm 1.20$; Challenge: $M = 4.28 \pm .74$; Self-Concept: $M = 3.89 \pm 1.05$; Control Expectancy: $M = 4.66 \pm .82$), as compared to previous studies (Herhaus & Petrowski, 2018; Het, Rohleder, Schoofs, Kirschbaum, & Wolf, 2009). Furthermore, cortisol AUC_I of the TSST related significantly to primary scale Primary Appraisal, $r = .465$, $p < .001$, $N = 52$, and tertiary scale Stress Index, $r = .409$, $p < .01$, $N = 52$. In addition, ACTH AUC_I showed a significant association to primary scale Primary Appraisal, $r = .432$, $p < .001$, $N = 52$, and tertiary scale Stress Index, $r = .378$, $p < .01$, $N = 52$. In addition, a significant difference was

Table 4
Area under the Curve with Respect to Increase for Cortisol and ACTH of Low/High DEX-CRH Responders in the TSST and the DEX-CRH Test

		M (*SD*)				
		Cortisol AUC_I TSST	Cortisol AUC_I DEX-CRH	*t*(25)	*p*	factor: TSST to DEX-CRH
DEX-CRH Responder (*n* = 52)	Low (*n* = 26)	513.56 (316.99)	13.43 (17.36)	8.012	< .001*** (*d* = 2.23)	38
	High (*n* = 26)	243.86 (152.69)	325.40 (419.79)	0.911	.371	1
		ACTH AUC_I TSST	ACTH AUC_I DEX-CRH			
DEX-CRH Responder (*n* = 52)	Low (*n* = 26)	7.35 (7.76)	1.16 (1.85)	2.180	.078[a]	6
	High (*n* = 26)	5.41 (8.11)	5.54 (4.60)	−3.490	< .01**[a] (*d* = −.97)	1

Note. AUC_I = area under the curve with respect to increase; DEX-CRH = dexamethasone-corticotropin releasing hormone test; TSST = Trier Social Stress Test.
[a] Calcuation based on logarithmized data. ** $p < .01$. *** $p < .001$.

Table 5
Row and Column Totals and Percentages Values for TSST Responding by DEX-CRH Responding

		TSST Responder	
		Low	High
DEX-CRH-Responder	Low	11 (21%)	15 (29%)
	High	15 (29%)	11 (21%)

Total $N = 52$.
$x^2 = .267$, $df = 1$, $p = .27$.
Note. DEX-CRH = dexamethasone-corticotropin releasing hormone test; TSST = Trier Social Stress Test.

observed between TSST low and high responders in the primary scale Threat, $t(25) = -2.358$, $p < .05$, $d = -0.65$, and the secondary scale Primary Appraisal, $t(25) = -2.363$, $p < .05$, $d = -0.66$, of the PASA, with higher values in the high responders.

Results in Table 5 indicate a higher percentage of overlap in the subgroups of high TSST and low DEX-CRH responders as well as high DEX-CRH and low TSST responders, but there was no significant difference in the distribution of responder groups regarding the two tests, $\chi^2 = .267$, $df = 1$, $p = .27$. When analyzing the cortisol reactivity of the subgroup TSST high responders/DEX-CRH low responders and of the subgroup DEX-CRH high responders/TSST low responders, there was significantly higher cortisol reactivity of the TSST high responders/DEX-CRH low responders as compared to DEX-CRH high responders/TSST low responders in the TSST, Cortisol AUC_I: $t(28) = 8.7006$, $p < .001$, $d = 3.18$. In contrast, DEX-CRH high responders/TSST low responders demonstrated significant higher cortisol reactivity compared to the TSST high responders/DEX-CRH low responders in the DEX-CRH test, Cortisol AUC_I: $t(28) = -6.017$, $p < .001$, $d = -2.20$.

Discussion

The DEX-CRH test and the TSST have been used in numerous studies for the activation of the HPA axis. To our knowledge, only the comparison study by Kirschbaum et al. (1993b) simultaneously implemented and compared the cortisol reactivity of healthy participants between CRH injection and TSST. They demonstrated similar cortisol peak values after psychological stress and bolus injection of 100 μg CRH, but different temporal patterns. The present study compared the differentiated hormonal responses to the standardized psychosocial stress test (TSST) and the standardized pharmacological challenge test (DEX-CRH) as well as the interaction of the responsiveness between both tests.

Note that both tests assess different aspects of stress responsiveness and activate the HPA axis in different ways. The DEX/CRH test is a paradigm used to test glucocorticoid negative feedback whereas the TSST is a test that evaluates the responsiveness of the axis. Consequently, a difference in time and amplitude of the hormonal stress response is expected, as DEX/CRH aims at suppressing the HPA axis, which induces an attenuated response of the pituitary and adrenal to CRH. The TSST, on the other hand, is a psychosocial stressor which activates central responses as well as the resultant HPA activation. However, despite targeting different mechanisms in stress reactivity, it is not clear in which way there might be an association between the centrally induced stress reactivity and the attenuation of stress responsiveness via glucocorticoid feedback.

Accordingly, both tests led to a distinct increase in the cortisol and the ACTH concentration. Significant differences were observed in the total cortisol increase and the timing of the cortisol peak. After psychosocial stress induction, the healthy participants showed a 3 times greater increase of cortisol compared to the DEX-CRH test.

Regarding the ACTH concentration, the healthy participants showed significantly higher values after the TSST and reached their highest value earlier. However, when the differences in the starting points were taken into consideration, the amount of increase and the later recovery phase in ACTH did not differ between the two tests. Therefore, the amplitude was identical. In contrast to the similar response pattern in ACTH, the cortisol showed a greater increase in TSST than in DEX-CRH, even when the starting point was taken into consideration.

Taken together, the stronger activation of the HPA axis due to psychosocial stress in comparison to the pharmacological challenge test DEX-CRH confirms a successful suppression of HPA axis activity via negative glucocorticoid feedback. As in the previous study comparing the CRH test and TSST (Kirschbaum et al., 1993b), both groups of healthy subjects showed similar maximums in cortisol concentration; in fact, one might argue that the 3 times lower increase in the DEX-CRH test resulted from the negative feedback loop. Interestingly, in the CRH test (Kirschbaum et al., 1993b) as well as in the DEX-CRH test, the

maximum cortisol concentration was reached later compared to the TSST-challenge, pointing to quicker response of stress-related higher order cognitive processes compared to the fundamental neuroendocrine feedback loops.

Although speculative, it may be relevant to elucidate potential physiological mechanisms underlying the different reactivity patterns in both tests. Despite application of dexamethasone, cortisol baseline levels are comparable across groups and showed no significant differences. This can be interpreted in the sense of dexamethasone mainly affecting the reactivity to stress as opposed to basal cortisol levels. The glucocorticoid receptor mediated inhibition of stress-reactivity occurs at numerous central nervous system nodes and may result, for example, in lower ACTH levels after CRH stimulation in the DEX-CRH test. The comparable levels of ACTH after both challenge tests associated with significantly different cortisol levels probably demonstrate dissociation between ACTH and glucocorticoid release, which has been shown in numerous species (Bornstein, Engeland, Ehrhart-Bornstein, & Herman, 2008). In the DEX-CRH test, dexamethasone may decrease the sensitivity of melanocortin 2 receptors in the zona fasciculata, which are the primary target of ACTH at the adrenal cortex resulting in lower cortisol levels. However, it is likely that this is not the only mechanism explaining the diverging cortisol response to similar ACTH levels in the two groups. Regarding the TSST, there is evidence from studies with rodents (Ulrich-Lai & Engeland, 2002) as well as with patients in the context of surgical stress (Gibbison et al., 2015) and critical illness (Boonen et al., 2014) that an activation of the sympathetic nervous system can result in cortisol secretion without a corresponding increase in ACTH. This can be partially mediated by cytokines (Bethin, Vogt, & Muglia, 2000). Therefore, the dissociation of ACTH and the cortisol response in the TSST may mirror the impact of the sympathoadrenomedullary system on the adrenal cortex, which may stimulate cortisol secretion as well as adrenal ACTH sensitivity. This mechanism mirrors the close interaction between the HPA axis and sympathetic nervous system probably orchestrated by higher brain centers in providing an adequate stress response. From an evolutionary perspective, the stress response is of great importance to ensure survival in changing environments. To be able to guarantee survival, it is highly important for the organism to distinguish between different kinds of stressors to adapt to the environment in the best possible way. This differentiation ensures that activation of the HPA axis is modulated based on the appraisal of the stressor (Herman et al., 2016). This means that the degree of activation of the HPA mirrors the degree of perceived threat. In this way, the organism can preserve resources by limiting the energetically costly activation of the HPA. There is broad evidence that inappropriate or prolonged HPA axis activation is linked to numerous physiological and psychological disease states. The dissociation of the ACTH and the cortisol response, as outlined earlier, may be one neurophysiological pathway facilitating this differentiation.

As opposed to this state-dependent stress response, the different hormonal response patterns in the subgroups may be seen as trait-dependent, reflecting an interindividually different sensitivity to anxiety and anxiety-related hormones corresponding to a highly specific reactivity of the HPA axis. Thus, the extent of cortisol increase after the TSST compared to the DEX-CRH test differed taking into account our subgroup analysis. High TSST responders revealed significantly higher cortisol levels compared to their levels in the DEX-CRH test. A similar pattern could be seen in low DEX-CRH responders. These significant differences in cortisol levels are not associated with significantly different ACTH levels in respective subgroups reflecting the aforementioned dissociation between the two hormones. Regarding the overlap between high and low responders to the TSST and DEX-CRH test, our findings descriptively showed a greater percentage of overlap between high DEX-CRH and low TSST responders and high TSST and low DEX-responders. However, there was no significant difference, probably due to the small sample size for testing between-subjects effects. These findings may be interpreted as support for our assumption that a higher cortisol-stress reaction in the TSST is associated with a higher susceptibility to the suppression of cortisol release by negative glucocorticoid feedback. On the other hand, of those individuals whose cortisol release is particularly responsive to negative glucocorticoid feedback, a higher percentage may also be more responsive to the HPA axis activation by a psychosocial stressor. This points to a high responsiveness of the stress axis in these subgroups, which can be regarded as an important physiological mechanism for the ability to deal with stress.

In contrast, low TSST responders and high DEX-CRH responders exhibited no difference in cortisol reactivity between the two stress tests. The low cortisol response to the TSST resembles the reaction pattern of anxious patients (Petrowski, Herold, Joraschky, Wittchen, & Kirschbaum,

2010). Respective patients have difficulties in recovering from the stressor. There is evidence that different reactivity patterns of the cortisol response are associated with a dysregulation of the HPA axis (Coryell, Noyes, & Reich, 1991; Goldberg, 1980; Holsboer, Liebl, & Hofschuster, 1982; Petrowski et al., 2013); however, the pathophysiological implications in terms of the development or manifestation of (mental) disease are still unclear. A low cortisol response in healthy subjects also may point to coping strategies within the framework of anticipatory cognitive appraisal (Gaab, Rohleder, Nater, & Ehlert, 2005). Additionally, the cortisol reactivity pattern of DEX-CRH high responders is still within the range of healthy subjects of other studies and shows no connection to diseases (Erhardt et al., 2006; Rybakowski & Twardowska, 1999). However, the present data suggest that there may be a possible association of DEX-CRH high responders and TSST low responders to the development of abnormalities of the HPA axis. Further research with the inclusion of additional parameters, such as pro-anti-inflammatory cytokines, feedback loop, and glucocorticoid sensitivity may pave the way toward a clearer understanding of the complex pathophysiology.

From a clinical viewpoint, the question may be posed whether the greater increase in the hormonal parameter cortisol by a factor of 3 after psychosocial stress induction compared to the pharmacological challenge test DEX-CRH also applies to patients and whether specific patient groups might exhibit different reactivity profiles. It is well-known that patients suffering from major depression show higher values in the cortisol response to the DEX-CRH test (Mokhtari, Arfken, & Boutros, 2013) and equal or higher values in the TSST (Wichmann et al., 2017; Young et al., 2004), as compared to healthy individuals. On the other hand, patients with panic disorder show a decreased cortisol response as compared to healthy individuals under psychosocial stress induction (Petrowski et al., 2013) and an intravenous injection of 100 µg CRH (Petrowski et al., 2012). The diagnosis of pathological stress reactivity requires an exact analysis regarding the extent of deviation of the hormonal response to psychosocial and pharmacological stress induction between patients and healthy participants. To obtain a clearer picture about a higher or lower stress reaction, the individually different hormonal reactions must be taken into consideration as to their norm values and progressions. In reference to different stress-related and psychological disorders, which have a different dysregulation of the HPA axis, there is growing evidence that a valid diagnostic procedure can be obtained only with the two types of stimulation tests discussed. This assessment is also supported by Modell and Holsboer (2006), who additionally showed that in some cases, the combination of a social stressor such as the TSST with the DEX-CRH test is more useful than the DEX-CRH test alone. They based this on a long-term investigation of over 10 years in which participants with a high familial risk for affective disorders showed abnormal results in the DEX-CRH test for the first time after a psychological stressor was experienced (Modell et al., 1998).

The strengths of this study are, first, the use of the standardized safe, reproducible, endocrinological pharmacological challenge test (DEX-CRH test) and the standardized psychosocial stress test (TSST); second, methodological procedures with regard to the same time window of both tests, control of confounders in the cortisol, and ACTH measurements (e.g., menstrual cycle); and third, the large sample size regarding the two hypotheses.

Several limitations also should be noted in the current study. A limiting factor lies in the mechanism of stress induction with the TSST lacking previous cortisol suppression via dexamethasone. Furthermore, the order of TSST and DEX-CRH challenges was fixed and not randomized. Finally, it must be considered that for testing the overlap between the subgroups (between-subjects effects), the sample size was too small.

In sum, we investigated stress reactivity using the DEX/-CRH test, which is a paradigm for testing glucocorticoid negative feedback as well as the TSST, which evaluates HPA responsiveness to psychosocial stress. Induction of psychosocial stress showed a greater increase by factor 3 in cortisol compared to the pharmacological challenge test DEX-CRH in healthy test participants without a similar increase in ACTH, which underlines dissociation between ACTH and cortisol stress responsiveness. Furthermore, we identified subgroups, which showed that a higher proportion of individuals with a blunted cortisol-stress reaction in the TSST also show lower susceptibility to the suppression of cortisol release by negative glucocorticoid feedback. On the other hand, a higher proportion of those individuals, in which cortisol release is less responsive to negative glucocorticoid feedback, is less responsive to the HPA axis activation by a psychosocial stressor. These results suggest an interaction between responsiveness in the TSST and the DEX-CRH test regarding cortisol patterns. Particularly, a blunted cortisol response to a psychosocial stressor may be

seen as a clinically relevant HPA axis dysfunction, which can also be found in anxiety disorders.

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

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